

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 14 April 2000 (14.04.00)	in its capacity as elected Office
International application No. PCT/GB99/03057	Applicant's or agent's file reference PHM 70389/WO
International filing date (day/month/year) 15 September 1999 (15.09.99)	Priority date (day/month/year) 19 September 1998 (19.09.98)
Applicant	
MORTEN, John, Edward, Norris	

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

17 March 2000 (17.03.00)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

<p>The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No.: (41-22) 740.14.35</p>	<p>Authorized officer</p> <p>Pascal Piriou</p> <p>Telephone No.: (41-22) 338.83.38</p>
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PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) PHM 70389/WO

Box No. I TITLE OF INVENTION

CHEMICAL COMPOUNDS

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

ZENECA Limited
15 Stanhope Gate
LONDON
GB-W1Y 6LN
GB

This person is also inventor.

Telephone No.
(01625) 516173

Faximile No.
(01625) 583358

Teleprinter No.
669095/669388

State (that is, country) of nationality:
GB

State (that is, country) of residence:
GB

This person is applicant all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box for the purposes of:

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

MORTEN, John Edward Norris
Alderley Park
Macclefield
Cheshire GB-SK10 4TG
GB

This person is:

applicant only

applicant and inventor

inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:
GB

State (that is, country) of residence:
GB

This person is applicant all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box for the purposes of:

Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

agent common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

GILES, Allen Frank
Global Intellectual Property
ASTRAZENECA PLC
Mereside, Alderley Park
Macclesfield, Cheshire, GB-SK10 4TG, GB

Telephone No.

(01625) 516573

Faximile No.

(01625) 583358

Teleprinter No.

669095/669388

Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):

Regional Patent

AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line):

<input checked="" type="checkbox"/> AL Albania	<input checked="" type="checkbox"/> LS Lesotho
<input checked="" type="checkbox"/> AM Armenia	<input checked="" type="checkbox"/> LT Lithuania
<input checked="" type="checkbox"/> AT Austria	<input checked="" type="checkbox"/> LU Luxembourg
<input checked="" type="checkbox"/> AU Australia	<input checked="" type="checkbox"/> LV Latvia
<input checked="" type="checkbox"/> AZ Azerbaijan	<input checked="" type="checkbox"/> MD Republic of Moldova
<input checked="" type="checkbox"/> BA Bosnia and Herzegovina	<input checked="" type="checkbox"/> MG Madagascar
<input checked="" type="checkbox"/> BB Barbados	<input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia
<input checked="" type="checkbox"/> BG Bulgaria	<input checked="" type="checkbox"/> MN Mongolia
<input checked="" type="checkbox"/> BR Brazil	<input checked="" type="checkbox"/> MW Malawi
<input checked="" type="checkbox"/> BY Belarus	<input checked="" type="checkbox"/> MX Mexico
<input checked="" type="checkbox"/> CA Canada	<input checked="" type="checkbox"/> NO Norway
<input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein	<input checked="" type="checkbox"/> NZ New Zealand
<input checked="" type="checkbox"/> CN China	<input checked="" type="checkbox"/> PL Poland
<input checked="" type="checkbox"/> CU Cuba	<input checked="" type="checkbox"/> PT Portugal
<input checked="" type="checkbox"/> CZ Czech Republic	<input checked="" type="checkbox"/> RO Romania
<input checked="" type="checkbox"/> DE Germany	<input checked="" type="checkbox"/> RU Russian Federation
<input checked="" type="checkbox"/> DK Denmark	<input checked="" type="checkbox"/> SD Sudan
<input checked="" type="checkbox"/> EE Estonia	<input checked="" type="checkbox"/> SE Sweden
<input checked="" type="checkbox"/> ES Spain	<input checked="" type="checkbox"/> SG Singapore
<input checked="" type="checkbox"/> FI Finland	<input checked="" type="checkbox"/> SI Slovenia
<input checked="" type="checkbox"/> GB United Kingdom	<input checked="" type="checkbox"/> SK Slovakia
<input checked="" type="checkbox"/> GD Grenada	<input checked="" type="checkbox"/> SL Sierra Leone
<input checked="" type="checkbox"/> GE Georgia	<input checked="" type="checkbox"/> TJ Tajikistan
<input checked="" type="checkbox"/> GH Ghana	<input checked="" type="checkbox"/> TM Turkmenistan
<input checked="" type="checkbox"/> GM Gambia	<input checked="" type="checkbox"/> TR Turkey
<input checked="" type="checkbox"/> HR Croatia	<input checked="" type="checkbox"/> TT Trinidad and Tobago
<input checked="" type="checkbox"/> HU Hungary	<input checked="" type="checkbox"/> UA Ukraine
<input checked="" type="checkbox"/> ID Indonesia	<input checked="" type="checkbox"/> UG Uganda
<input checked="" type="checkbox"/> IL Israel	<input checked="" type="checkbox"/> US United States of America
<input checked="" type="checkbox"/> IN India	<input checked="" type="checkbox"/> UZ Uzbekistan
<input checked="" type="checkbox"/> IS Iceland	<input checked="" type="checkbox"/> VN Viet Nam
<input checked="" type="checkbox"/> JP Japan	<input checked="" type="checkbox"/> YU Yugoslavia
<input checked="" type="checkbox"/> KE Kenya	<input checked="" type="checkbox"/> ZW Zimbabwe
<input checked="" type="checkbox"/> KG Kyrgyzstan	
<input checked="" type="checkbox"/> KP Democratic People's Republic of Korea	
<input checked="" type="checkbox"/> KR Republic of Korea	
<input checked="" type="checkbox"/> KZ Kazakhstan	
<input checked="" type="checkbox"/> LC Saint Lucia	
<input checked="" type="checkbox"/> LK Sri Lanka	
<input checked="" type="checkbox"/> LR Liberia	

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

DM Dominica

AE United Arab Emirates

ZA South Africa

CR Costa Rica

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

Box No. VI PRIORITY CLAIM		<input type="checkbox"/> Further priority claims are indicated in the Supplemental Box.		
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country	regional application: regional Office	international application: receiving Office
item (1) 19-Sep-98 (19.09.98)	9820338.3	GB		
item (2)				
item (3)				

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s):

* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA)
(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):

ISA /

Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):

Date (day/month/year) Number Country (or regional Office)

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following number of sheets:

request	:	3
description (excluding sequence listing part)	:	16
claims	:	2
abstract	:	1
drawings	:	
sequence listing part of description	:	1

Total number of sheets : 23

This international application is accompanied by the item(s) marked below:

1. fee calculation sheet
2. separate signed power of attorney
3. copy of general power of attorney; reference number, if any:
4. statement explaining lack of signature
5. priority document(s) identified in Box No. VI as item(s):
6. translation of international application into (language):
7. separate indications concerning deposited microorganism or other biological material
8. nucleotide and/or amino acid sequence listing in computer readable form
9. other (specify):

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

GILES, Allen Frank
AGENT FOR APPLICANT

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings:	
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	<input type="checkbox"/> received:	<input type="checkbox"/> not received:
4. Date of timely receipt of the required corrections under PCT Article 11(2):		
5. International Searching Authority (if two or more are competent): ISA /	6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid.	

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

See Notes to the request form

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year) 27 October 2000 (27.10.00)
Applicant's or agent's file reference PHM 70389/WO
International application No. PCT/GB99/03057

From the INTERNATIONAL BUREAU

To:

GILES, Allen, Frank
AstraZeneca
Global Intellectual Property
P.O. Box 272
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4GR
ROYAUME-UNI

IMPORTANT NOTIFICATION

International filing date (day/month/year)
15 September 1999 (15.09.99)

1. The following indications appeared on record concerning:

the applicant the inventor the agent the common representative

Name and Address ASTRAZENECA UK LIMITED 15 Stanhope Gate London W1Y 6LN United Kingdom	State of Nationality GB	State of Residence GB
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person the name the address the nationality the residence

Name and Address ASTRAZENECA AB S-151 85 Södertälje Sweden	State of Nationality SE	State of Residence SE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Christine Carrié Telephone No.: (41-22) 338.83.38
---	--

PATENT COOPERATION TREATY

AFG

COPY TO DEG

PCT

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S 06 NOV 2000

ASTRAZENECA
GLOBAL INTELLECTUAL PROPERTYNOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year)

27 October 2000 (27.10.00)

Applicant's or agent's file reference

PHM 70389/WO

International application No.

PCT/GB99/03057

From the INTERNATIONAL BUREAU

To:

GILES, Allen, Frank
AstraZeneca
Global Intellectual Property
P.O. Box 272
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4GR
ROYAUME-UNI

IMPORTANT NOTIFICATION

1. The following indications appeared on record concerning:

 the applicant the inventor the agent the common representative

Name and Address

ASTRAZENECA UK LIMITED
15 Stanhope Gate
London W1Y 6LN
United Kingdom

State of Nationality

GB

State of Residence

GB

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

 the person the name the address the nationality the residence

Name and Address

ASTRAZENECA AB *OV*
S-151 85 Södertälje
Sweden

State of Nationality

SE

State of Residence

SE

Telephone No.

Facsimile No.

Teleprinter No.

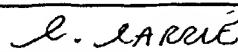
3. Further observations, if necessary:

4. A copy of this notification has been sent to:

 the receiving Office
 the International Searching Authority
 the International Preliminary Examining Authority

 the designated Offices concerned
 the elected Offices concerned
 other:
The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer


 Christine Carrié

Facsimile No.: (41-22) 740.14.35

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year)

27 October 2000 (27.10.00)

Applicant's or agent's file reference

PHM 70389/WO

IMPORTANT NOTIFICATION

International application No.

PCT/GB99/03057

International filing date (day/month/year)

15 September 1999 (15.09.99)

1. The following indications appeared on record concerning:

the applicant the inventor the agent the common representative

Name and Address

GILES, Allen, Frank
AstraZeneca
Global Intellectual Property
Mereside, Alderley Park
Macclesfield
Cheshire SK10 4TG
United Kingdom

State of Nationality

State of Residence

Telephone No.

01625/516573

Facsimile No.

01625/583358

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person the name the address the nationality the residence

Name and Address

GILES, Allen, Frank
AstraZeneca
Global Intellectual Property
P.O. Box 272
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4GR
United Kingdom

State of Nationality

State of Residence

Telephone No.

01625 514304

Facsimile No.

01625 583358

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

 the receiving Office the International Searching Authority the International Preliminary Examining Authority the designated Offices concerned the elected Offices concerned other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Christine Carrié

Telephone No.: (41-22) 338.83.38

AFG

RECEIVED

- 5 MAY 2000

ASIF - L'ENCLAVE
GLOBAL INTELLECTUAL PROPERTY

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)Date of mailing (day/month/year)
25 April 2000 (25.04.00)

From the INTERNATIONAL BUREAU

To:

GILES, Allen, Frank
AstraZeneca
Global Intellectual Property
Mereside, Alderley Park
Macclesfield
Cheshire SK10 4TG
ROYAUME-UNIApplicant's or agent's file reference
PHM 70389/WO

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/03057International filing date (day/month/year)
15 September 1999 (15.09.99)

1. The following indications appeared on record concerning:

 the applicant the inventor the agent the common representative

Name and Address

ZENECA LIMITED
15 Stanhope Gate
London W1Y 6LN
United Kingdom

State of Nationality

GB

State of Residence

GB

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

 the person the name the address the nationality the residence

Name and Address

ASTRAZENECA UK LIMITED
15 Stanhope Gate
London W1Y 6LN
United Kingdom

State of Nationality

GB

State of Residence

GB

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

 the receiving Office
 the International Searching Authority
 the International Preliminary Examining Authority

 the designated Offices concerned
 the elected Offices concerned
 other:

Authorized officer

Sean Taylor

SAS

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Telephone No.: (41-22) 338.83.38

Facsimile No.: (41-22) 740.14.35

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PHM 70389/WO	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 99/ 03057	International filing date (day/month/year) 15/09/1999	(Earliest) Priority Date (day/month/year) 19/09/1998
Applicant ZENECA LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

contained in the international application in written form.

filed together with the international application in computer readable form.

furnished subsequently to this Authority in written form.

furnished subsequently to this Authority in computer readable form.

the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. **Certain claims were found unsearchable (See Box I).**

3. **Unity of invention is lacking (see Box II).**

4. With regard to the **title**,

the text is approved as submitted by the applicant.

the text has been established by this Authority to read as follows:

POLYMORPHISMS IN THE HUMAN VCAM-1 GENE, SUITABLE FOR DIAGNOSIS AND TREATMENT OF VCAM-1 LIGAND MEDIATED DISEASES

5. With regard to the **abstract**,

the text is approved as submitted by the applicant.

the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

as suggested by the applicant.

because the applicant failed to suggest a figure.

because this figure better characterizes the invention.

—

None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03057

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 G06F17/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WENZEL K ET AL.: "DNA polymorphisms in adhesion molecule genes - a new risk factor for early atherosclerosis" HUMAN GENETICS, vol. 97, 1996, pages 15-20, XP000867269 abstract ---	1,3,5, 8-10
Y	NEWTON C R ET AL: "ANALYSIS OF ANY POINT MUTATION IN DNA. THE AMPLIFICATION REFRACTORY MUTATION SYSTEM (ARMS)" NUCLEIC ACIDS RESEARCH, GB, OXFORD UNIVERSITY PRESS, SURREY, vol. 17, no. 7, page 2503-2516 XP000141596 ISSN: 0305-1048 the whole document --- -/-	1,3

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

*** Special categories of cited documents :**

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

24 January 2000

Date of mailing of the international search report

04/02/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl
Fax: (+31-70) 340-3016

Authorized officer

Knehr, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03057

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92 00751 A (NOVONORDISK AS) 23 January 1992 (1992-01-23) abstract; claims 18-29 ----	8-10
Y	WO 97 49731 A (ZENECA LTD ;DUTTA ANAND SWAROOP (GB)) 31 December 1997 (1997-12-31) cited in the application abstract page 29, line 19 -page 31, line 12; claims 17-21 ----	8,10
Y	WO 97 40462 A (SPECTRA BIOMEDICAL INC) 30 October 1997 (1997-10-30) the whole document ----	5
A	IADEMARCO M F ET AL.: "Characterization of the promotor for vascular cell adhesion molecule-1 (VCAM-1)" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 267, no. 23, 1992, pages 16323-16329, XP002128420 cited in the application the whole document ----	
A	LIN K-C AND CASTRO A C: "Very late antigen 4 (VLA4) antagonists as anti-inflammatory agents" CURRENT OPINION IN CHEMICAL BIOLOGY, vol. 2, 1998, pages 453-457, XP000869619 cited in the application the whole document -----	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 99/03057

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 9 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/03057

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9200751	A 23-01-1992	AU 8205591	A	04-02-1992
WO 9749731	A 31-12-1997	AU 3102797	A	14-01-1998
		EP 0910582	A	28-04-1999
		HR 970338	A	30-04-1998
		NO 985966	A	18-12-1998
WO 9740462	A 30-10-1997	AU 2734197	A	12-11-1997
		EP 0897567	A	24-02-1999

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 99/03057

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 9 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03057

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 G06F17/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category [°]	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WENZEL K ET AL.: "DNA polymorphisms in adhesion molecule genes - a new risk factor for early atherosclerosis" HUMAN GENETICS, vol. 97, 1996, pages 15-20, XP000867269 abstract ---	1,3,5, 8-10
Y	NEWTON C R ET AL: "ANALYSIS OF ANY POINT MUTATION IN DNA. THE AMPLIFICATION REFRACTORY MUTATION SYSTEM (ARMS)" NUCLEIC ACIDS RESEARCH, GB, OXFORD UNIVERSITY PRESS, SURREY, vol. 17, no. 7, page 2503-2516 XP000141596 ISSN: 0305-1048 the whole document --- -/-	1,3

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

24 January 2000

04/02/2000

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Knehr, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/03057

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92 00751 A (NOVONORDISK AS) 23 January 1992 (1992-01-23) abstract; claims 18-29 ----	8-10
Y	WO 97 49731 A (ZENECA LTD ;DUTTA ANAND SWAROOP (GB)) 31 December 1997 (1997-12-31) cited in the application abstract page 29, line 19 -page 31, line 12; claims 17-21 ----	8,10
Y	WO 97 40462 A (SPECTRA BIOMEDICAL INC) 30 October 1997 (1997-10-30) the whole document ----	5
A	IADEMARCO M F ET AL.: "Characterization of the promotor for vascular cell adhesion molecule-1 (VCAM-1)" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 267, no. 23, 1992, pages 16323-16329, XP002128420 cited in the application the whole document ----	
A	LIN K-C AND CASTRO A C: "Very late antigen 4 (VLA4) antagonists as anti-inflammatory agents" CURRENT OPINION IN CHEMICAL BIOLOGY, vol. 2, 1998, pages 453-457, XP000869619 cited in the application the whole document -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/03057

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9200751 A	23-01-1992	AU	8205591 A	04-02-1992
WO 9749731 A	31-12-1997	AU	3102797 A	14-01-1998
		EP	0910582 A	28-04-1999
		HR	970338 A	30-04-1998
		NO	985966 A	18-12-1998
WO 9740462 A	30-10-1997	AU	2734197 A	12-11-1997
		EP	0897567 A	24-02-1999

PATENT COOPERATION TREATY

PCT

REC'D 21 SEP 2000	
WIPO	PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PHM.70389/WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB99/03057	International filing date (day/month/year) 15/09/1999	Priority date (day/month/year) 19/09/1998
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant ASTRAZENECA UK LIMITED et al.		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 8 sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input checked="" type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application 		

Date of submission of the demand 17/03/2000	Date of completion of this report 19.09.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Hoesel, H Telephone No. +49 89 2399 8693



INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

International application No. PCT/GB99/03057

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-16 as originally filed

Claims, No.:

1-11 as originally filed

2. The amendments have resulted in the cancellation of:

the description, pages:
 the claims, Nos.:
 the drawings, sheets:

3. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

the entire international application.
 claims Nos. 5, 9.

because:

the said international application, or the said claims Nos. 5, 9 relate to the following subject matter which does not require an international preliminary examination (*specify*):

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/03057

see separate sheet

- the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

- the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

- no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims 1 - 3, 6, 7, 11
	No: Claims 4, 8 - 10
Inventive step (IS)	Yes: Claims
	No: Claims 1 - 4, 6 - 11
Industrial applicability (IA)	Yes: Claims 1 - 4, 6 - 8, 10, 11
	No: Claims

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03057

Reference is made to the following documents:

D1: WO-A-97/49731

D2: Lin & Castro, Current Opinion in Chem. Biol. vol. 2, 1998, p. 453-7

D3: Wenzel et al, Hum. Gen. vol. 97, 1996, p. 15 - 20

D4: Iademarco et al, J.Biol. Chem. vol. 267, 1992, p. 16323-29

SECTION I:

1. The sequence listing as originally filed consisting of one page has been also taken into consideration as a basis for the present report.

SECTION III:

2. Claim 5 relates to a "computer readable medium" having stored thereon some sequence information. This subject-matter falls under "presentation of information" in the sense of Rule 67.1(v) PCT. Consequently, no opinion will be formulated with respect to novelty, inventive step and industrial applicability of this subject-matter (Article 34(4)(a)(i) PCT).
3. Claim 9 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

SECTION V:

4. Claims 8 - 10 broadly relate to the medical use of VCAM-1 ligand antagonists (in the preparation of a medicament, in the therapy, as part of a pharmaceutical pack).
 - 4.1. As also indicated in the description, such medicaments and their therapeutical uses are known in the state of the art (some are mentioned in the abstracts of D1 and D2).

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03057

4.2. The medical indication given in claim 8 is unusual and obscure (see Section VIII) and additionally cannot be distinguished from medical indications for VCAM-1 ligand antagonists known or suggested in the prior art of D1 and D2.

Thus, the medical use according to claim 8 lacks novelty and inventive step (Art. 33(2) and (3) PCT) with respect to each of D1 and D2.

4.3. The same applies to the methods of treatment as defined in claim 9; definition in terms of how a diagnosis is obtained is irrelevant for the method of therapy. The said feature cannot therefore be taken into consideration for assessment of novelty and inventive step of claim 9.

4.3. The presence of written instructions (claim 10) is not a technical feature and thus not suitable to establish novelty and inventive step with respect to known pharmaceutical agents, the more, as it is in this case not apparent by which features the intended therapy can be distinguished from those applied in the prior art.

Having regard to the relevant technical features, i.e. the compounds to be administrated, pharmaceutical packs according to claim 10 cannot be distinguished from other pharmaceutical products containing VCAM-1 antagonist drugs. Thus, claim 10 lacks novelty and inventive step in view of D1 (Art. 33(2) and (3) PCT).

5. Due to the use of the non-limiting wording "comprising", claim 4 extends to any genomic DNA obtained from tissue specimens of individuals accidentally having the said base exchanges (whether they are diagnosed to have them or not).

Due to its breadth, the claim is considered to lack novelty (Art. 33(2) PCT).

6. The present application concerns polymorphisms in the promoter region of the VCAM-1 gene and nucleic acid suitable for potential diagnostic application (claims 1 - 3, 4, 6 and 7).

The closest prior art for this subject-matter results from D3. The document concerns the identification of polymorphisms in various genes coding for cell

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03057

adhesion molecules, including VCAM-1, in patients suffering from cardiovascular disease. Polymorphisms in the coding region as well as in the 5' flanking, untranslated region have been investigated. Only 3 alleles, all located in the E-selectin gene, out of a number of 17 investigated polymorphisms could be correlated with increased risk for early atherosclerosis (see D3, the abstract, table 1, p. 19, col. 1 lines, 15 - 23 and lines 42 - 54). The investigation as to whether further VCAM-1 polymorphisms show a disease correlation, e.g. with atherosclerosis is suggested in D3 (p. 19, col. 1, lines 9 - 23 and 42 - 45) and thus obvious.

The contribution to the prior art of D3 by claims 1 - 3 resides in the identification of further VCAM-1 alleles. The application, however, fails to provide evidence as to a disease correlation of any of the discovered VCAM-1 alleles. Thus, the method of claims 1 - 3 lacks an unexpected, beneficial technical effect.

Consequently, the subject-matter of claims 1 - 3 lacks inventive step, contrary to Art. 33(3) PCT in view of D3. This analogously applies to the use according to claim 11.

7. In the absence of a defined technical effect, the sequences of claim 4, the primers and probes probe according to claims 6 and 7 represent equivalents to known VCAM-1 sequences, such as known primers and probes that are capable of detecting (silent) VCAM-1 polymorphisms (cf. D3), contrary to Art. 33(3) PCT.

Alternatively, the sequences covered by claims 4, 6 and 7, may be considered as discoveries without technical character and not as an invention as defined in the PCT-Guidelines C-IV, 1.2(ii).

SECTION VII:

8. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D3 and D4 is not mentioned in the description, nor are these documents identified therein.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03057

9. The application is not self-contained in that the relevant promoter sequence has been defined only in terms of the EMBL accession number. No additional sequence listing concerning the said nucleic acid has been submitted (Rule 13ter, 1(a) and (f) PCT).

SECTION VIII:

10. If claims 1 - 3 are to be interpreted as to concern the diagnosis of particular, defined diseases, the following is noted:

Contrary to polymorphisms in the coding region, nucleotide changes in the promoter region will not result in changes of the primary and/or secondary structure of the gene product. Base alterations in the 5' flanking sequences may change the expression of the gene product, but only, if they are located in regulatory domains and if they effect on the interaction capability of the regulatory domains. In other words, many if not most potential point mutations in 5' flanking sequences are expected to be silent (cf. the abstract of D3).

As mentioned above, the application fails to provide any evidence for an allele specific change in the VCAM-1 expression, or a correlation of allele frequency with increased risk for one particular disease. It is, in this connection, noted that the point mutations are located at positions -1902, -1534, -1474, 1433, -1352 and -714 upstream the transcription initiation site, i.e. outside the conventional transcription regulation elements and, furthermore, all except one outside the regions indicated as potential regulatory elements in D4 (see fig. 3).

Therefore, in the absence of evidence on the contrary it is expected that the identified polymorphisms are not accompanied with an altered transcription or translation efficiency.

Thus, if claim is to be interpreted as to concern the diagnosis of a particular disease with one of the discovered nucleic acid exchanges, the claim is not supported and, in the absence of such a correlation, not enabled, contrary to Art. 5 and 6 PCT.

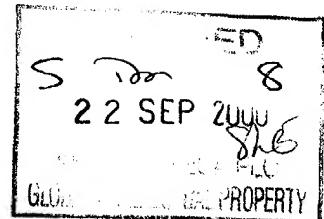
**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03057

11. The medical indication given in claim "for treating a VCAM-mediated disease in a human **diagnosed as having a...**" is obscure. It furthermore leaves the skilled reader in doubt whether and how VCAM-1 ligand mediated diseases expressed by the selected group of patients differ from those known in the state of the art (Art. 6 PCT).
12. Claim 9 is directed to the method of therapy but is defined in features that are irrelevant for such subject-matter (step (i)). Their presence raises uncertainty as to the scope and category of the claim (Art. 6. PCT).

PATENT COOPERATION TREATY

PCT



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PHM.70389/WO	FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB99/03057	International filing date (day/month/year) 15/09/1999	Priority date (day/month/year) 19/09/1998	
International Patent Classification (IPC) or national classification and IPC C12Q1/68			
Applicant ASTRAZENECA UK LIMITED et al.			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 17/03/2000	Date of completion of this report 19.09.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Hoesel, H Telephone No. +49 89 2399 8693



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/03057

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-16 as originally filed

Claims, No.:

1-11 as originally filed

2. The amendments have resulted in the cancellation of:

the description, pages:
 the claims, Nos.:
 the drawings, sheets:

3. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

the entire international application.
 claims Nos. 5, 9.

because:

the said international application, or the said claims Nos. 5, 9 relate to the following subject matter which does not require an international preliminary examination (*specify*):

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/03057

see separate sheet

- the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

- the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

- no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1 - 3, 6, 7, 11
	No:	Claims 4, 8 - 10
Inventive step (IS)	Yes:	Claims
	No:	Claims 1 - 4, 6 - 11
Industrial applicability (IA)	Yes:	Claims 1 - 4, 6 - 8, 10, 11
	No:	Claims

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03057

Reference is made to the following documents:

D1: WO-A-97/49731

D2: Lin & Castro, Current Opinion in Chem. Biol. vol. 2, 1998, p. 453-7

D3: Wenzel et al, Hum. Gen. vol. 97, 1996, p. 15 - 20

D4: Iademarco et al, J.Biol. Chem. vol. 267, 1992, p. 16323-29

SECTION I:

1. The sequence listing as originally filed consisting of one page has been also taken into consideration as a basis for the present report.

SECTION III:

2. Claim 5 relates to a "computer readable medium" having stored thereon some sequence information. This subject-matter falls under "presentation of information" in the sense of Rule 67.1(v) PCT. Consequently, no opinion will be formulated with respect to novelty, inventive step and industrial applicability of this subject-matter (Article 34(4)(a)(i) PCT).
3. Claim 9 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

SECTION V:

4. Claims 8 - 10 broadly relate to the medical use of VCAM-1 ligand antagonists (in the preparation of a medicament, in the therapy, as part of a pharmaceutical pack).
 - 4.1. As also indicated in the description, such medicaments and their therapeutical uses are known in the state of the art (some are mentioned in the abstracts of D1 and D2).

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03057

4.2. The medical indication given in claim 8 is unusual and obscure (see Section VIII) and additionally cannot be distinguished from medical indications for VCAM-1 ligand antagonists known or suggested in the prior art of D1 and D2.

Thus, the medical use according to claim 8 lacks novelty and inventive step (Art. 33(2) and (3) PCT) with respect to each of D1 and D2.

4.3. The same applies to the methods of treatment as defined in claim 9; definition in terms of how a diagnosis is obtained is irrelevant for the method of therapy. The said feature cannot therefore be taken into consideration for assessment of novelty and inventive step of claim 9.

4.3. The presence of written instructions (claim 10) is not a technical feature and thus not suitable to establish novelty and inventive step with respect to known pharmaceutical agents, the more, as it is in this case not apparent by which features the intended therapy can be distinguished from those applied in the prior art.

Having regard to the relevant technical features, i.e. the compounds to be administrated, pharmaceutical packs according to claim 10 cannot be distinguished from other pharmaceutical products containing VCAM-1 antagonist drugs. Thus, claim 10 lacks novelty and inventive step in view of D1 (Art. 33(2) and (3) PCT).

5. Due to the use of the non-limiting wording "comprising", claim 4 extends to any genomic DNA obtained from tissue specimens of individuals accidentally having the said base exchanges (whether they are diagnosed to have them or not).

Due to its breadth, the claim is considered to lack novelty (Art. 33(2) PCT).

6. The present application concerns polymorphisms in the promoter region of the VCAM-1 gene and nucleic acid suitable for potential diagnostic application (claims 1 - 3, 4, 6 and 7).

The closest prior art for this subject-matter results from D3. The document concerns the identification of polymorphisms in various genes coding for cell

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03057

adhesion molecules, including VCAM-1, in patients suffering from cardiovascular disease. Polymorphisms in the coding region as well as in the 5' flanking, untranslated region have been investigated. Only 3 alleles, all located in the E-selectin gene, out of a number of 17 investigated polymorphisms could be correlated with increased risk for early atherosclerosis (see D3, the abstract, table 1, p. 19, col. 1 lines, 15 - 23 and lines 42 - 54). The investigation as to whether further VCAM-1 polymorphisms show a disease correlation, e.g. with atherosclerosis is suggested in D3 (p. 19, col. 1, lines 9 - 23 and 42 - 45) and thus obvious.

The contribution to the prior art of D3 by claims 1 - 3 resides in the identification of further VCAM-1 alleles. The application, however, fails to provide evidence as to a disease correlation of any of the discovered VCAM-1 alleles. Thus, the method of claims 1 - 3 lacks an unexpected, beneficial technical effect.

Consequently, the subject-matter of claims 1 - 3 lacks inventive step, contrary to Art. 33(3) PCT in view of D3. This analogously applies to the use according to claim 11.

7. In the absence of a defined technical effect, the sequences of claim 4, the primers and probes probe according to claims 6 and 7 represent equivalents to known VCAM-1 sequences, such as known primers and probes that are capable of detecting (silent) VCAM-1 polymorphisms (cf. D3), contrary to Art. 33(3) PCT.

Alternatively, the sequences covered by claims 4, 6 and 7, may be considered as discoveries without technical character and not as an invention as defined in the PCT-Guidelines C-IV, 1.2(ii).

SECTION VII:

8. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D3 and D4 is not mentioned in the description, nor are these documents identified therein.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03057

9. The application is not self-contained in that the relevant promoter sequence has been defined only in terms of the EMBL accession number. No additional sequence listing concerning the said nucleic acid has been submitted (Rule 13ter, 1(a) and (f) PCT).

SECTION VIII:

10. If claims 1 - 3 are to be interpreted as to concern the diagnosis of particular, defined diseases, the following is noted:

Contrary to polymorphisms in the coding region, nucleotide changes in the promoter region will not result in changes of the primary and/or secondary structure of the gene product. Base alterations in the 5' flanking sequences may change the expression of the gene product, but only, if they are located in regulatory domains and if they effect on the interaction capability of the regulatory domains. In other words, many if not most potential point mutations in 5' flanking sequences are expected to be silent (cf. the abstract of D3).

As mentioned above, the application fails to provide any evidence for an allele specific change in the VCAM-1 expression, or a correlation of allele frequency with increased risk for one particular disease. It is, in this connection, noted that the point mutations are located at positions -1902, -1534, -1474, 1433, -1352 and -714 upstream the transcription initiation site, i.e. outside the conventional transcription regulation elements and, furthermore, all except one outside the regions indicated as potential regulatory elements in D4 (see fig. 3).

Therefore, in the absence of evidence on the contrary it is expected that the identified polymorphisms are not accompanied with an altered transcription or translation efficiency.

Thus, if claim is to be interpreted as to concern the diagnosis of a particular disease with one of the discovered nucleic acid exchanges, the claim is not supported and, in the absence of such a correlation, not enabled, contrary to Art. 5 and 6 PCT.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03057

11. The medical indication given in claim "for treating a VCAM-mediated disease in a human **diagnosed as having a...**" is obscure. It furthermore leaves the skilled reader in doubt whether and how VCAM-1 ligand mediated diseases expressed by the selected group of patients differ from those known in the state of the art (Art. 6 PCT).
12. Claim 9 is directed to the method of therapy but is defined in features that are irrelevant for such subject-matter (step (i)). Their presence raises uncertainty as to the scope and category of the claim (Art. 6. PCT).

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(71) Applicant (for all designated States except US): ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB).			
(72) Inventor; and (75) Inventor/Applicant (for US only): MORTEN, John, Edward, Norris [GB/GB]; Alderley Park, Macclefield, Cheshire SK10 4TG (GB).		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
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(54) Title: POLYMORPHISMS IN THE HUMAN VCAM-1 GENE, SUITABLE FOR DIAGNOSIS AND TREATMENT OF VCAM-1 LIGAND MEDIATED DISEASES			
(57) Abstract <p>This invention relates to polymorphisms in the human Vascular Cell Adhesion Molecule-1 (VCAM-1) gene, in particular at one or more of positions 278, 647, 707, 748, 829 and 1467 in the VCAM-1 gene as defined by the positions in EMBL ACCESSION NO. M92431. The invention also relates to methods and materials for analysing allelic variation in the VCAM-1 gene, and to the use of VCAM-1 polymorphism in the diagnosis and treatment of VCAM-1 ligand mediated diseases such as multiple sclerosis, rheumatoid arthritis, atherosclerosis and allergic asthma.</p>			

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POLYMORPHISMS IN THE HUMAN VCAM-1 GENE, SUITABLE FOR DIAGNOSIS AND TREATMENT OF VCAM-1 LIGAND MEDIATED DISEASES

This invention relates to polymorphisms in the human Vascular Cell Adhesion Molecule-1 (VCAM-1) gene. The invention also relates to methods and materials for analysing allelic variation in the VCAM-1 gene, and to the use of VCAM-1 polymorphism in the diagnosis and treatment of VCAM-1 ligand mediated diseases such as multiple sclerosis, rheumatoid arthritis, atherosclerosis and allergic asthma.

VCAM-1, also known as CD106, is a 90-110 kDa glycoprotein member of the immunoglobulin superfamily expressed mainly on the surface of activated vascular endothelial cells. Two forms of human VCAM-1 have been identified, a predominant form containing seven immunoglobulin domains and an alternatively-spliced form missing the fourth immunoglobulin domain. VCAM-1 is also found as a soluble form in serum, probably as a result of proteolytic cleavage of endothelial cell surface VCAM-1. Cell adhesion molecules have been reviewed in Mousa *et al.* (1997), DDT, 2, 187-199.

VCAM-1 is a ligand for the α_4 integrins, $\alpha_4\beta_1$, also known as Very Late Antigen-4 (VLA-4) or CD49d/CD29, and $\alpha_4\beta_7$. These integrins are members of a family of heterodimeric cell surface receptors that are composed of non-covalently associated glycoprotein subunits (α and β) and are involved in the adhesion of cells to other cells or to extracellular matrix. Integrin $\alpha_4\beta_1$ is expressed on numerous haematopoietic cells, including haematopoietic precursors, peripheral and cytotoxic T lymphocytes, B lymphocytes, monocytes, thymocytes and eosinophils. Integrin $\alpha_4\beta_7$ is expressed on lymphocytes that preferentially home to gastrointestinal mucosa and gut-associated lymphoid tissue. The α_4 integrins recognise a short amino acid sequence, glutamine-isoleucine-aspartic acid-serine-proline (QIDSP), exposed on the C-D loop of immunoglobulin domains 1 and 4. An accessory binding site may also be located in the adjacent immunoglobulin domain.

Expression of VCAM-1 on unactivated vascular endothelial cells is low or absent but is upregulated in human inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, allergic asthma and atherosclerosis. Soluble VCAM-1 is elevated in serum and cerebrospinal fluid of multiple sclerosis patients and in serum during inflammatory bowel disease and after cardiac transplantation. *In vitro*, endothelial cells can be induced to express VCAM-1 by inflammatory cytokines such as tumour necrosis factor and interleukin-1 or by oxidative stress. VCAM-1 gene expression in vascular endothelial cells during inflammation is regulated by

transcriptional activation, thought to involve the dimeric transcription factor, nuclear factor- κ B (NF- κ B).

The activation and extravasation of blood leukocytes plays a major role in the development and progression of inflammatory diseases. Cell adhesion to the vascular 5 endothelium is required before cells migrate from the blood into inflammed tissue and is mediated by specific interactions between cell adhesion molecules on the surface of vascular endothelial cells and circulating leukocytes. α_4 integrin/VCAM-1 binding is believed to have an important role in the recruitment of lymphocytes, monocytes and eosinophils during inflammation. Monoclonal antibodies directed against the α_4 integrin subunit have been 10 shown to be effective in a number of animal models of human inflammatory diseases including multiple sclerosis, rheumatoid arthritis, allergic asthma, contact dermatitis, transplant rejection, insulin-dependent diabetes, inflammatory bowel disease, and glomerulonephritis.

$\alpha_4\beta_1$ /VCAM-1 binding has also been implicated in T-cell proliferation, B-cell localisation to germinal centres, haematopoietic progenitor cell localisation in the bone 15 marrow, angiogenesis, placental development, muscle development and tumour cell metastasis.

Small molecule inhibitors of VCAM-1 binding to α_4 integrins have been designed based on the QIDSP motif in VCAM-1 and similar motifs in other α_4 integrin ligands fibronectin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1). Small molecule and monoclonal antibody inhibitors of VCAM-1 binding to α_4 integrins and inhibitors of VCAM-1 expression 20 may have utility in the treatment of autoimmune, allergic and vascular inflammatory diseases, the prevention of tumour metastasis and in mobilisation of haematopoietic progenitor cells from bone marrow prior to tumour chemotherapy.

Exon 1 of the VCAM-1 gene has been cloned and published as a EMBL Accession number: M92431 (2396 bp) and all positions herein relate to the position therein unless stated 25 otherwise or apparent from the context.

One approach is to use knowledge of polymorphisms to help identify patients most suited to therapy with particular pharmaceutical agents (this is often termed "pharmacogenetics"). Pharmacogenetics can also be used in pharmaceutical research to assist the drug selection process. Polymorphisms are used in mapping the human genome and to 30 elucidate the genetic component of diseases. The reader is directed to the following references for background details on pharmacogenetics and other uses of polymorphism detection: Linder *et al.* (1997), Clinical Chemistry, 43, 254; Marshall (1997), Nature Biotechnology, 15,

1249; International Patent Application WO 97/40462, Spectra Biomedical; and Schafer *et al.* (1998), *Nature Biotechnology*, **16**, 33.

Clinical trials have shown that patient response to treatment with pharmaceuticals is often heterogeneous. Thus there is a need for improved approaches to pharmaceutical agent 5 design and therapy.

The present invention is based on the discovery of six single nucleotide polymorphisms (SNPs) in the VCAM-1 gene.

According to one aspect of the present invention there is provided a method for the diagnosis of a single nucleotide polymorphism in VCAM-1 in a human, which method 10 comprises determining the sequence of the nucleic acid of the human at one or more of positions 278, 647, 707, 748, 829 and 1467 in the VCAM-1 gene as defined by the positions in EMBL ACCESSION NO. M92431, and determining the status of the human by reference to polymorphism in the VCAM-1 gene.

The term human includes both a human having or suspected of having a VCAM-1 15 ligand mediated disease and an asymptomatic human who may be tested for predisposition or susceptibility to such disease. At each position the human may be homozygous for an allele or the human may be a heterozygote.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 278 is presence of T 20 and/or C.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 647 is presence of A and/or G.

In another embodiment of the invention preferably the method for diagnosis described 25 herein is one in which the single nucleotide polymorphism at position 707 is presence of T and/or C.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 748 is presence of T and/or C.

30 In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 829 is presence of G and/or A.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 1467 is presence of T and/or C.

The method for diagnosis is preferably one in which the sequence is determined by a 5 method selected from amplification refractory mutation system and restriction fragment length polymorphism.

In another aspect of the invention we provide a method for the diagnosis of VCAM-1 ligand-mediated disease, which method comprises:

- i) obtaining sample nucleic acid from an individual,
- 10 ii) detecting the presence or absence of a variant nucleotide at one or more of positions 278, 647, 707, 748, 829 and 1467 (as defined by the position in EMBL accession number M92431), in the VCAM-1 gene and
- iii) determining the status of the individual by reference to polymorphism in the VCAM-1 gene.

Allelic variation at position 278 consists of a single base substitution from T (the 15 published base), preferably to C. Allelic variation at position 647 consists of a single base substitution from A (the published base), preferably to G. Allelic variation at position 707 consists of a single base substitution from T (the published base), preferably to C. Allelic variation at position 748 consists of a single base substitution from T (the published base), preferably to C. Allelic variation at position 829 consists of a single base substitution from G 20 (the published base), preferably to A. Allelic variation at position 1467 consists of a single base substitution from T (the published base), preferably to C. The status of the individual may be determined by reference to allelic variation at any one, two, three, four, five or all six positions.

The test sample of nucleic acid is conveniently a sample of blood, bronchoalveolar 25 lavage fluid, sputum, or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before analysis of allelic variation.

It will be apparent to the person skilled in the art that there are a large number of 30 analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification

reaction and optionally a signal generation system. Table 1 lists a number of mutation detection techniques, some based on the PCR. These may be used in combination with a number of signal generation systems, a selection of which is listed in Table 2. Further amplification techniques are listed in Table 3. Many current methods for the detection of 5 allelic variation are reviewed by Nollau *et al.*, Clin. Chem. **43**, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

Abbreviations:

ALEX™	Amplification refractory mutation system linear extension
APEX	Arrayed primer extension
ARMS™	Amplification refractory mutation system
b-DNA	Branched DNA
CMC	Chemical mismatch cleavage
bp	base pair
COPS	Competitive oligonucleotide priming system
DGGE	Denaturing gradient gel electrophoresis
FRET	Fluorescence resonance energy transfer
LCR	Ligase chain reaction
MAdCAM-1	mucosal addressin cell adhesion molecule-1
MASDA	Multiple allele specific diagnostic assay
NASBA	Nucleic acid sequence based amplification
OLA	Oligonucleotide ligation assay
PCR	Polymerase chain reaction
PTT	Protein truncation test
RFLP	Restriction fragment length polymorphism
SDA	Strand displacement amplification
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism analysis
SSR	Self sustained replication
TGGE	Temperature gradient gel electrophoresis
VCAM-1	Vascular Cell Adhesion Molecule-1
VLA-4	Very Late Antigen-4

10 Table 1 - Mutation Detection Techniques

General: DNA sequencing, Sequencing by hybridisation

Scanning: PTT*, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage

* Note: not useful for detection of promoter polymorphisms.

15 Hybridisation Based

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips)

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, 14, 303; WO 5 95/13399 (Public Health Inst., New York)

Extension Based: ARMSTM, ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, 17, 2347.

Incorporation Based: Mini-sequencing, APEX

Restriction Enzyme Based: RFLP, Restriction site generating PCR

10 **Ligation Based:** OLA

Other: Invader assay

Table 2 - Signal Generation or Detection Systems

Fluorescence: FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)

15 **Other:** Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric, Hybridisation protection assay, Mass spectrometry

Table 3 - Further Amplification Methods

SSR, NASBA, LCR, SDA, b-DNA

Preferred mutation detection techniques include ARMSTM, ALEX™, COPS, Taqman, 20 Molecular Beacons, RFLP, and restriction site based PCR and FRET techniques.

Particularly preferred methods include ARMSTM and RFLP based methods. ARMSTM is an especially preferred method.

In a further aspect, the diagnostic methods of the invention are used to assess the 25 efficacy of therapeutic compounds in the treatment of VCAM-1 ligand mediated diseases such as autoimmune, allergic and vascular inflammatory diseases. The polymorphisms identified in the present invention occur in the promoter region of the VCAM-1 gene. The changes are not expected to alter the amino acid sequence of VCAM-1, but several of the polymorphisms affect transcription sites within the promoter region and thus may affect the transcription of the VCAM-1 gene. For example the changing of the nucleotide at position 748 (as defined by the 30 position in EMBL ACCESSION NO. M92431) from T to C results in the gain of a E1a-F rev site and the loss of a TATA box.

Assays, for example reporter-based assays, may be devised to detect whether one or more of the above polymorphisms affect transcription levels and/or message stability.

Individuals who carry particular allelic variants of the VCAM-1 gene may therefore exhibit differences in their ability to regulate protein biosynthesis under different physiological conditions and will display altered abilities to react to different diseases. In addition, differences in protein regulation arising as a result of allelic variation may have a direct effect on the response of an individual to drug therapy. The diagnostic methods of the invention may be useful both to predict the clinical response to such agents and to determine therapeutic dose.

10 In a further aspect, the diagnostic methods of the invention, are used to assess the predisposition and/or susceptibility of an individual to diseases mediated by VCAM-1 ligands. This may be particularly relevant in the development of autoimmune, allergic and vascular inflammatory diseases and other diseases which are modulated by VCAM-1 interactions. The present invention may be used to recognise individuals who are particularly at risk from 15 developing these conditions.

15 In a further aspect, the diagnostic methods of the invention are used in the development of new drug therapies which selectively target one or more allelic variants of the VCAM-1 gene. Identification of a link between a particular allelic variant and predisposition to disease development or response to drug therapy may have a significant impact on the design of new 20 drugs. Drugs may be designed to regulate the biological activity of variants implicated in the disease process whilst minimising effects on other variants.

25 In a further diagnostic aspect of the invention the presence or absence of variant nucleotides is detected by reference to the loss or gain of, optionally engineered, sites recognised by restriction enzymes. In the accompanying Example 1 we provide details of a convenient engineered restriction enzyme sites that are lost or gained as a result of a polymorphism of the invention.

According to another aspect of the present invention there is provided a nucleic acid comprising any one of the following polymorphisms:

the nucleic acid of EMBL ACCESSION No. M92431 with C at position 278 in the promoter 30 sequence as defined by the position in EMBL ACCESSION No. M92431; the nucleic acid of EMBL ACCESSION No. M92431 with G at position 647 in the promoter sequence as defined by the position in EMBL ACCESSION No. M92431;

the nucleic acid of EMBL ACCESSION No. M92431 with C at position 707 in the promoter sequence as defined by the position in EMBL ACCESSION No. M92431;

the nucleic acid of EMBL ACCESSION No. M92431 with C at position 748 in the promoter sequence as defined by the position in EMBL ACCESSION No. M92431;

5 the nucleic acid of EMBL ACCESSION No. M92431 with A at position 829 in the promoter sequence as defined by the position in EMBL ACCESSION No. M92431;

the nucleic acid of EMBL ACCESSION No. M92431 with C at position 1467 in the promoter sequence as defined by the position in EMBL ACCESSION No. M92431;

or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at 10 least one polymorphism.

Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases. The nucleic acid of the invention does not encompass naturally occurring nucleic acid as it occurs in nature, for example, the nucleic acid is at least partially purified from at least one component with which it occurs naturally. Preferably the nucleic acid is at 15 least 30% pure, more preferably at least 60% pure, more preferably at least 90% pure, more preferably at least 95% pure, and more preferably at least 99% pure.

According to another aspect of the invention there is provided use of a nucleic acid sequence comprising at least one of the polymorphisms in the promoter disclosed herein to identify compounds that modify expression of the VCAM-1 gene. Modification of expression 20 includes inhibition or enhancement of expression. This is conveniently done by measuring expression levels of a reporter gene (for example beta-galactosidase) under the control of the promoter in transfected host cells in the presence or absence of test compounds. Suitable test compounds include polynucleotides capable of binding to the promoter through triplex strand formation. Accordingly suitable compounds can be identified for therapeutic use which alter 25 native gene expression either up or down as appropriate for the relevant disease to be treated. The reader is directed to the following references on nucleic acid triplex formation and uses: Progress in developments of Triplex-Based strategies: Giovannangeli C; Helene C: Antisense and Nucleic Acid Drug Development / 7/4 (413-421) /1997; Recent developments in triple-helix regulation of gene expression: Neidle S: Anti-Cancer Drug Design / 12/5 (433-442) 30 /1997; Triplex DNA: Fundamentals, advances, and potential applications for gene therapy: Chan PP; Glazer PM: Journal of Molecular Medicine / 75/4 (267-282) /1997; Oligonucleotide directed triple helix formation: Sun J-S; Garestier T; Helene C: Current Opinion in Structural

Biology / 6/3 (327-333) /1996; C Mayfield, M Squibb, D Miller (1994) Inhibition of nuclear protein binding to the human Ki-ras promoter by triplex-forming oligonucleotides Biochemistry 33,3358-3363; WM Olivas, LJ Maher (1996) Binding of DNA oligonucleotides to sequences in the promoter of the human bcl-2 gene Nucleic Acids Research 24, 1758-1764; 5 C Mayfield, S Ebinghaus, J Gees, D Jones, B Rodu, M Squibb, D Miller (1994) Triplex formation by the human HA-ras promoter inhibits Sp1 binding and in vitro transcription J Biol Chem 269,18232-18238; and JE Gee, GR Revankar, TS Rao, ME Hogan (1995) Triplex formation at the rat neu gene utilizing imidazole and 2'-deoxy-6-thioguanosine base substitutions Biochemistry 34,2042-2048.

10 According to another aspect of the present invention there is provided a computer readable medium comprising at least one novel polynucleotide sequence of the invention stored on the medium. The computer readable medium may be used, for example, in homology searching, mapping, haplotyping, genotyping or pharmacogenetic analysis or any other bioinformatic analysis. The reader is referred to Bioinformatics, A practical guide to the 15 analysis of genes and proteins, Edited by A D Baxevanis & B F F Ouellette, John Wiley & Sons, 1988. Any computer readable medium may be used, for example, compact disk, tape, floppy disk, hard drive or computer chips.

The polynucleotide sequences of the invention, or parts thereof, particularly those relating to and identifying the single nucleotide polymorphisms identified herein represent a 20 valuable information source, for example, to characterise individuals in terms of haplotype and other sub-groupings, such as investigation of susceptibility to treatment with particular drugs. These approaches are most easily facilitated by storing the sequence information in a computer readable medium and then using the information in standard bioinformatics programs or to search sequence databases using state of the art searching tools such as "GCC". Thus, the 25 polynucleotide sequences of the invention are particularly useful as components in databases useful for sequence identity and other search analyses. As used herein, storage of the sequence information in a computer readable medium and use in sequence databases in relation to 'polynucleotide or polynucleotide sequence of the invention' covers any detectable chemical or physical characteristic of a polynucleotide of the invention that may be reduced to, 30 converted into or stored in a tangible medium, such as a computer disk, preferably in a computer readable form. For example, chromatographic scan data or peak data, photographic scan or peak data, mass spectrographic data, sequence gel (or other) data.

The invention provides a computer readable medium having stored thereon one or a more polynucleotide sequences of the invention. For example, a computer readable medium is provided comprising and having stored thereon a member selected from the group consisting of: a polynucleotide comprising the sequence of a polynucleotide of the invention, a 5 polynucleotide consisting of a polynucleotide of the invention, a polynucleotide which comprises part of a polynucleotide of the invention, which part includes at least one of the polymorphisms of the invention, a set of polynucleotide sequences wherein the set includes at least one polynucleotide sequence of the invention, a data set comprising or consisting of a polynucleotide sequence of the invention or a part thereof comprising at least one of the 10 polymorphisms identified herein. A computer based method is also provided for performing sequence identification, said method comprising the steps of providing a polynucleotide sequence comprising a polymorphism of the invention in a computer readable medium; and comparing said polymorphism containing polynucleotide sequence to at least one other polynucleotide or polypeptide sequence to identify identity (homology), i.e. screen for the 15 presence of a polymorphism.

The invention further provides nucleotide primers which can detect the polymorphisms of the invention.

According to another aspect of the present invention there is provided an allele specific primer capable of detecting a VCAM-1 gene polymorphism at one or more of positions 278, 20 647, 707, 748, 829 and 1467 in the VCAM-1 gene as defined by the positions in EMBL ACCESSION NO. M92431.

An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as 25 used for ARMS™ assays. The allele specific primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

An allele specific primer preferably corresponds exactly with the allele to be detected but derivatives thereof are also contemplated wherein about 6-8 of the nucleotides at the 3' terminus correspond with the allele to be detected and wherein up to 10, such as up to 8, 6, 4, 30 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the primer.

Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If 5 required the primer(s) may be labelled to facilitate detection.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a VCAM-1 gene polymorphism at one or more of positions 278, 647, 707, 748, 829 and 1467 in the VCAM-1 gene as defined by the positions in EMBL ACCESSION NO. M92431.

10 The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In 15 general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection.

According to another aspect of the present invention there is provided a diagnostic kit 20 comprising an allele specific oligonucleotide probe of the invention and/or an allele-specific primer of the invention.

The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase.

25 In another aspect of the invention, the single nucleotide polymorphisms of this invention may be used as genetic markers in linkage studies. This particularly applies to the polymorphism at 278 (as defined by the position in EMBL ACCESSION NO. M92431) because of its relatively high frequency (see below). The VCAM-1 gene has been mapped to chromosome 1p31-32 (Cybulsky et al Proc. Natl. Acad. Sci. USA **88**, 7859-7863, 1991).

30 Low frequency polymorphisms may be particularly useful for haplotyping as described below. A haplotype is a set of alleles found at linked polymorphic sites (such as within a gene) on a single (paternal or maternal) chromosome. If recombination within the gene is random,

there may be as many as 2^n haplotypes, where 2 is the number of alleles at each SNP and n is the number of SNPs. One approach to identifying mutations or polymorphisms which are correlated with clinical response is to carry out an association study using all the haplotypes that can be identified in the population of interest. The frequency of each haplotype is limited 5 by the frequency of its rarest allele, so that SNPs with low frequency alleles are particularly useful as markers of low frequency haplotypes. As particular mutations or polymorphisms associated with certain clinical features, such as adverse or abnormal events, are likely to be of low frequency within the population, low frequency SNPs may be particularly useful in identifying these mutations (for examples see: Linkage disequilibrium at the cystathionine beta 10 synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. *Ann Hum Genet* (1998) **62**:481-90, De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, and Gudnason V; and Variation at the von willebrand factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter. *Blood* 15 (1999) **93**:4277-83, Keightley AM, Lam YM, Brady JN, Cameron CL, Lillicrap D).

According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a VCAM-1 ligand antagonist drug in which the method comprises:

- i) diagnosis of a single nucleotide polymorphism in VCAM-1 gene in the human, which 20 diagnosis comprises determining the sequence of the nucleic acid at one or more of positions 278, 647, 707, 748, 829 and 1467 (as defined by the position in EMBL accession number M92431), and determining the status of the human by reference to polymorphism in the VCAM-1 gene; and
- ii) administering an effective amount of a VCAM-1 ligand antagonist .

25 Preferably determination of the status of the human is clinically useful. Examples of clinical usefulness include deciding which antagonist drug or drugs to administer and/or in deciding on the effective amount of the drug or drugs.

VCAM-1 ligand antagonist drugs have been disclosed in the following publications: international patent application WO 97/49731, Zeneca Limited; international patent application 30 WO 97/02289, Zeneca Limited; international patent application WO 96/20216, Zeneca Limited; US patent 5510332, Texas Biotechnology; international patent application WO 96/01644, Athena Neurosciences; international patent application WO 96/01644, Athena

Neurosciences and; international patent application WO 96/00581, Zeneca Limited. A VCAM-1 ligand antagonist drug may act directly at VCAM-1 and/or at a ligand, such as VLA-4, which binds to VCAM-1.

According to another aspect of the present invention there is provided use of a VCAM-5 1 ligand antagonist drug in preparation of a medicament for treating a VCAM-1 ligand mediated disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions 278, 647, 707, 748, 829 and 1467 (as defined by the position in EMBL accession number M92431).

According to another aspect of the present invention there is provided a pharmaceutical 10 pack comprising VCAM-1 ligand antagonist drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism at one or more of positions 278, 647, 707, 748, 829 and 1467 (as defined by the position in EMBL accession number M92431).

The invention will now be illustrated but not limited by reference to the following 15 Examples. All temperatures are in degrees Celsius.

In the Examples below, unless otherwise stated, the following methodology and materials have been applied.

AMPLITAQ™, available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

20 General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

Electropherograms were obtained in a standard manner: data was collected by ABI377 25 data collection software and the wave form generated by ABI Prism sequencing analysis (2.1.2).

Example 1

Identification of Polymorphisms

1. Methods

DNA Preparation

30 DNA was prepared from frozen blood samples collected in EDTA following protocol I (Molecular Cloning: A Laboratory Manual, p392, Sambrook, Fritsch and Maniatis, 2nd Edition, Cold Spring Harbor Press, 1989) with the following modifications. The thawed blood was

diluted in an equal volume of standard saline citrate instead of phosphate buffered saline to remove lysed red blood cells. Samples were extracted with phenol, then phenol/chloroform and then chloroform rather than with three phenol extractions. The DNA was dissolved in deionised water.

5 Template Preparation

Templates were prepared by PCR using the oligonucleotide primers and annealing temperatures set out below. The extension temperature was 72° and denaturation temperature 94°, each step was 1 minute. All reactions contained 1 mM MgCl₂. Generally 50 ng of genomic DNA was used in each reaction and subjected to 35 cycles of PCR.

Fragment	Forward Oligo	Reverse Oligo	Annealing Temp
22-1151	22-41	1131-1151	62°
595-1151	595-618	1131-1151	58°
1264-1804	1264-1286	1782-1804	58°

10

For dye-primer sequencing the forward primers were modified to include M13 forward sequence (ABI protocol P/N 402114, Applied Biosystems) at the 5' end of the oligonucleotide. DNA polymerase (AmpliTaq Gold™, Perkin Elmer Cetus) was used to generate products 595-1151 and 1264-1804.

15 Dye Primer Sequencing

Dye-primer sequencing using M13 forward and reverse primers was as described in the ABI protocol P/N 402114 for the ABI Prism™ dye primer cycle sequencing core kit with "AmpliTaq FS"™ DNA polymerase, modified in that the annealing temperature was 45° and DMSO was added to the cycle sequencing mix to a final concentration of 5 %.

20 The extension reactions for each base were pooled, ethanol/sodium acetate precipitated, washed and resuspended in formamide loading buffer.

4.25 % Acrylamide gels were run on an automated sequencer (ABI 377, Applied Biosystems).

2. Results

25 Novel Polymorphisms

Position ¹	Published ²	Variant	RFLP	Variant Allele Frequency	TF ³ Site Gain	TF ³ site Loss

-15-

278	T	C	loss of Vsp I	41/94	LF-A2 rev, HNF1rev, SBF-1 rev, phyA3 rev	AP-1 rev
647	A	G	engineered Pvu I (see Example 2)	1/82	none	HNF-5, ZRE 3, 4,&6, GR intron site 4
707	T	C	none	9/82	none	none
748	T	C	gain of Bst F5 I	2/82	E1a-F rev	TATA box
829	G	A	gain of Ksp 632 I	1/108	none	MalT
1467	T	C	gain of Rsa I	1/82	none	TEF- 1,EFII,S ph box, D10

¹As defined by the position in EMBL ACCESSION NO. M92431

²Iadermarco *et al.* J. Biol. Chem, 267, 16323-16329, 1992.

³TF = transcription factor

Allele frequency was determined in a European control population.

5

Example 2

Engineered restriction sites for detection of polymorphisms

Standard methodology can be used to detect the polymorphism at positions 647 (as 10 defined by the position in EMBL ACCESSION NO. M92431) based on the materials set out below.

Diagnostic Fragment	Forward primer	Reverse primer
518-669	518-540	648-669 Pvu I

Reverse primer sequence CCCAGAGGTCTTACAGCGAT (SEQ ID NO:1).

The product generated by these primers will include a Pvu I site, only if the diagnostic 15 fragment contained a G allele at position 647.

Sequence Listing Free Text

<223> Description of Artificial Sequence:PCR primer

CLAIMS

- 1 A method for the diagnosis of a single nucleotide polymorphism in VCAM-1 in a human, which method comprises determining the sequence of the nucleic acid of the human at one or more of positions 278, 647, 707, 748, 829 and 1467 in the VCAM-1 gene as defined by 5 the positions in EMBL ACCESSION NO. M92431, and determining the status of the human by reference to polymorphism in the VCAM-1 gene.
- 2 A method for diagnosis according to claim 1 in which the single nucleotide polymorphisms are further defined as:
the single nucleotide polymorphism at position 278 is presence of T and/or C;
10 the single nucleotide polymorphism at position 647 is presence of A and/or G;
the single nucleotide polymorphism at position 707 is presence of T and/or C;
the single nucleotide polymorphism at position 748 is presence of T and/or C;
the single nucleotide polymorphism at position 829 is presence of G and/or A; and
the single nucleotide polymorphism at position 1467 is presence of T and/or C.
- 15 3 A method for diagnosis according to claim 1 or 2 in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length polymorphism.
- 4 A nucleic acid comprising any one of the following polymorphisms:
the nucleic acid of EMBL ACCESSION No. M92431 with C at position 278 in the promoter 20 sequence as defined by the position in EMBL ACCESSION No. M92431;
the nucleic acid of EMBL ACCESSION No. M92431 with G at position 647 in the promoter sequence as defined by the position in EMBL ACCESSION No. M92431;
the nucleic acid of EMBL ACCESSION No. M92431 with C at position 707 in the promoter sequence as defined by the position in EMBL ACCESSION No. M92431;
25 the nucleic acid of EMBL ACCESSION No. M92431 with C at position 748 in the promoter sequence as defined by the position in EMBL ACCESSION No. M92431;
the nucleic acid of EMBL ACCESSION No. M92431 with A at position 829 in the promoter sequence as defined by the position in EMBL ACCESSION No. M92431;
the nucleic acid of EMBL ACCESSION No. M92431 with C at position 1467 in the promoter 30 sequence as defined by the position in EMBL ACCESSION No. M92431;
or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one polymorphism.

5 A computer readable medium comprising at least one nucleic acid sequence as defined in claim 4 stored on the medium.

6 An allele specific primer capable of detecting a VCAM-1 gene polymorphism at one or more of positions 278, 647, 707, 748, 829 and 1467 in the VCAM-1 gene as defined by the 5 positions in EMBL ACCESSION NO. M92431.

7 An allele-specific oligonucleotide probe capable of detecting a VCAM-1 gene polymorphism at one or more of positions 278, 647, 707, 748, 829 and 1467 in the VCAM-1 gene as defined by the positions in EMBL ACCESSION NO. M92431.

8 Use of a VCAM-1 ligand antagonist drug in preparation of a medicament for treating a 10 VCAM-1 ligand mediated disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions 278, 647, 707, 748, 829 and 1467 as defined by the position in EMBL accession number M92431.

9 A method of treating a human in need of treatment with a VCAM-1 ligand antagonist drug in which the method comprises:

15 i) diagnosis of a single nucleotide polymorphism in VCAM-1 gene in the human, which diagnosis comprises determining the sequence of the nucleic acid at one or more of positions 278, 647, 707, 748, 829 and 1467 as defined by the position in EMBL accession number M92431, and determining the status of the human by reference to polymorphism in the VCAM-1 gene; and

20 ii) administering an effective amount of a VCAM-1 ligand antagonist .

10 A pharmaceutical pack comprising VCAM-1 ligand antagonist drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism at one or more of positions 278, 647, 707, 748, 829 and 1467 as defined by the position in EMBL accession number M92431.

25 11 Use of a nucleic acid sequence comprising at least one of the polymorphisms in the promoter at one or more of positions 278, 647, 707, 748, 829 and 1467 as defined by the position in EMBL accession number M92431 to identify compounds that modify expression of the VCAM-1 gene.

- 1 -

SEQUENCE LISTING

<110> Zeneca Limited

5 <120> Chemical Compounds

<130> VCAM

<140>

10 <141>

<150> 9820338.3

<151> 1998-09-19

15 <160> 1

<170> PatentIn Ver. 2.1

<210> 1

20 <211> 22

<212> DNA

<213> Artificial Sequence

<220>

25 <223> Description of Artificial Sequence:PCR primer

<400> 1

cccgaggtc ctttacagcg at

22

30

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03057

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 G06F17/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>WENZEL K ET AL.: "DNA polymorphisms in adhesion molecule genes - a new risk factor for early atherosclerosis" HUMAN GENETICS, vol. 97, 1996, pages 15-20, XP000867269 abstract</p> <p>---</p> <p>NEWTON C R ET AL: "ANALYSIS OF ANY POINT MUTATION IN DNA. THE AMPLIFICATION REFRACTORY MUTATION SYSTEM (ARMS)" NUCLEIC ACIDS RESEARCH, GB, OXFORD UNIVERSITY PRESS, SURREY, vol. 17, no. 7, page 2503-2516 XP000141596 ISSN: 0305-1048 the whole document</p> <p>---</p> <p>---</p>	1,3,5, 8-10
Y		1,3

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

^a Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

24 January 2000

04/02/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Knehr, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03057

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92 00751 A (NOVONORDISK AS) 23 January 1992 (1992-01-23) abstract; claims 18-29 ----	8-10
Y	WO 97 49731 A (ZENECA LTD ; DUTTA ANAND SWAROOP (GB)) 31 December 1997 (1997-12-31) cited in the application abstract page 29, line 19 -page 31, line 12; claims 17-21 ----	8,10
Y	WO 97 40462 A (SPECTRA BIOMEDICAL INC) 30 October 1997 (1997-10-30) the whole document ----	5
A	IADEMARCO M F ET AL.: "Characterization of the promotor for vascular cell adhesion molecule-1 (VCAM-1)" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 267, no. 23, 1992, pages 16323-16329, XP002128420 cited in the application the whole document ----	
A	LIN K-C AND CASTRO A C: "Very late antigen 4 (VLA4) antagonists as anti-inflammatory agents" CURRENT OPINION IN CHEMICAL BIOLOGY, vol. 2, 1998, pages 453-457, XP000869619 cited in the application the whole document ----	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 99/03057

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 9 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/03057

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9200751	A 23-01-1992	AU 8205591	A	04-02-1992
WO 9749731	A 31-12-1997	AU 3102797	A	14-01-1998
		EP 0910582	A	28-04-1999
		HR 970338	A	30-04-1998
		NO 985966	A	18-12-1998
WO 9740462	A 30-10-1997	AU 2734197	A	12-11-1997
		EP 0897567	A	24-02-1999